

Evaluation of the Efficacy of Microneedling with Tacrolimus versus Calcipotriol Plus Betamethasone Dipropionate in Vitiligo Treatment

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ABSTRACT

Background: Vitiligo is a hypopigmented skin lesion of unknown etiology. Microneedling is a safe and successful approach in vitiligo treatment. Dermoscopy plays an essential role with regard to the differentiation between vitiligo from other hypopigmentary disorders. **Objective:** To evaluate the clinical efficacy of microneedling with tacrolimus versus calcipotriol plus betamethasone dipropionate (Cal/BD) in vitiligo treatment through assessing changes in the degree of repigmentation of the lesions clinically and by dermoscope.

Patients and Methods: This was a prospective clinical trial carried on forty patients with vitiligo. The studied patient were divided into two sides; Side A was treated with microneedling and topical calcipotriol (0.05 mg/g) plus betamethasone dipropionate (0.5 mg) ointment. Side B was treated with microneedling and topical tacrolimus (0.03%) ointment. The cases were followed up every month for 3 months after the termination of therapeutic sessions.

Results: Good response was recognized with right side (A) and left side (B) as regarding the Vitiligo Extent Tensity Index (VETI) scoring before versus after treatment in each treatment group ($P < 0.001$ for both). The combination with Cal/BD has a statistically higher outcome compared with the combination with tacrolimus ointment as revealed by dermoscope assessment. **Conclusion:** Microneedling combined with Cal/BD ointment or combined with tacrolimus ointment seems to be safe and effective therapeutic options in vitiligo treatment. Dermoscopically, the combination with Cal/BD has a statistically higher outcome compared with the combination with tacrolimus ointment.

Keywords: Vitiligo, Microneedling, Tacrolimus, Dermoscope, Repigmentation.

INTRODUCTION

Vitiligo is a hypopigmented skin lesion developed owing to autoimmunity, which damages epidermal and occasionally melanocytes with a subsequent loss of melanocyte functions⁽¹⁾. Several theories have been emerged to elucidate the pathogenesis of various forms of vitiligo as genetic background, autoimmune state, neurohormonal, cytotoxicity and oxidative stress theory⁽²⁾. Localized vitiligo could be described as hypopigmented macules restricted to a single area and may be further subclassified into focal, segmental and mucosal types. Generalized vitiligo comprises at least two body regions and could be further subclassified into acrofacial, vulgaris, universal and mixed types⁽³⁾.

Dermoscopy has an important function in the differentiation of vitiligo from different hypopigmented lesions and in the diagnosis of different forms of vitiligo⁽⁴⁾. In addition, it detect disease stability before vitiligo surgeries are concerned as perifollicular hyperpigmentation, marginal hyperpigmentation, and marginal reticular pigmentation while in active disease trichrome, polka dot, salt and pepper and comet tail appearance were frequent findings⁽⁵⁾.

Various therapeutic approaches are recommended for vitiligo, which involve topical steroids, topical calcineurin inhibitors (TCIs), vitamin D analogues, antioxidants, phototherapy, laser therapies, surgeries and combined therapy⁽³⁾. Topical preparations of immune suppressing medications comprising glucocorticoids (e.g., betamethasone) and calcineurin

inhibitors (e.g., tacrolimus) are considered to be first line vitiligo treatments⁽²⁾. TCIs cause the repigmentation of vitiligo via the stimulation of melanocyte proliferation and migration and melanin synthesis⁽⁶⁾.

Calcipotriene is a form of vitamin D known to have immunosuppressive and immunomodulating characteristics. Keratinocytes, melanocytes, fibroblasts and active immunological cells have receptors for 1,25 dihydroxy cholecalciferol⁽⁷⁾.

Defects in melanocyte and keratinocyte receptors within the lesions have been demonstrated to be associated with defective calcium uptake. The association of topical corticosteroids and calcipotriene is demonstrated to be more efficient than calcipotriene only^(7,8). Microneedling is a simple office approach which includes forming thousands of microclefs across the epidermis into the papillary dermis⁽⁹⁾. It mainly depends on the principle of neocollagenesis and neovascularization, which happens owing to the generation of several growth factors following needle piercing of the stratum corneum⁽¹⁰⁾. We aimed to assess the clinical efficacy of microneedling with tacrolimus vs Cal/BD in vitiligo treatment through assessing changes in the degree of repigmentation of the lesions clinically and by dermoscope.

PATIENTS AND METHODS

This was a prospective clinical trial carried on forty patients with vitiligo attending the outpatient clinic of Dermatology, Andrology and STDs Department of Mansoura University Hospital. Patients were classified into two sides; Side A (Right side): was treated with

topical calcipotriol (0.05 mg/g) plus betamethasone dipropionate (0.5 mg) ointment with microneedling and Side B (Left side); was treated with topical tacrolimus (0.03%) ointment with microneedling.

Inclusion Criteria:

- Patients with varying severity aged 18-60 years.
- Cases having bilateral more or less symmetrical lesions on any site of the body.
- Cases who didn't receive topical or systemic therapy for the past 3 months.
- Different body sites were treated (face, neck, upper and lower limbs, trunk and acral areas).

Exclusion Criteria:

- Coagulations and bleeding disorders or on anticoagulants.
- Acute infections and hepatic disorders.
- Pregnancy and lactation.
- Keloid tendency.

All the patients were subjected to history taking, which included age, onset, duration of the disease, risk factors, potential causes, preceding treatments including history of previous phototherapy, and family history of similar condition. Complete general and dermatological examination was performed to assess any accompanying skin diseases and any signs of systemic disorders. Examination of vitiligo lesions was done by Wood's lamp to detect type of lesions and distribution.

Vitiligo Extent Tensity Index (VETI) scoring system⁽¹¹⁾ was used to assess the degree of vitiligo by a numerical score and combines analysis of extensity and severity of vitiligo and produce a constant number based on the rule of nine. Five areas affected, head (h), upper limbs (u), trunk (t), lower limbs (l) and genitalia (g) are individually scored by using five stages of disease tensity (T). Stage zero indicates normal skin, stage I indicates hypopigmentation, stage II indicates complete depigmentation with black hair and with perifollicular pigmentation, stage III indicates full depigmentation with black hair (no perifollicular pigmentation), stage IV indicates complete depigmentation with a mixture of white and black hair, in presence or absence of perifollicular pigmentation, while stage V indicates complete depigmentation combined with noticeable hair whitening.

The total body VETI is measured utilizing the next formula, which involves participations from all body areas: VETI score: (ratio of head affection × grade of tensity)+(ratio of trunk affection × grade of tensity) 4 + (ratio of upper limbs affection × grade of tensity) 2 +(ratio of lower limbs affection × grade of tensity) 4 + (ratio of genitalia affection × grade of tensity) 0.1⁽¹¹⁾.

Dermoscopic evaluation of vitiligo severity was done using cutoff scoring system using the "BPLFoSK criteria"⁽⁵⁾: 1-Sharp border (+1), 2-Pigment network within the patch (absent/reticulate) +1, 3-Perilesional

hyperpigmentation +1, 4-Perifollicular hyperpigmentation +1, 5-Satellite lesion -1.5, 6-MicroKobner phenomenon -2⁽⁵⁾.

Dermoscopy assessment was conducted using HUD **Dermlite** dermatoscope and images were obtained with **Galaxy Grand 2** camera connected to the dermatoscope with adapter. Laboratory investigations included CBC, bleeding and coagulation time, liver and renal function tests and TSH were done before treatment.

Examination of all cases were performed in the first visit and reviewed every 14 days to assess the progress of therapy and existence of adverse events and for sessions of microneedling. Patients were photographed at first visit clinically and dermoscopically and every session clinically until the termination of therapy and the last session were assessed clinically and dermoscopically that ensured our treatment. All the side effects were noticed and documented. Follow-up evaluation was done to determine stabilization or reactivation of vitiligo within a period of three months following the termination of therapy.

Ethical consideration:

The study design was approved by the Ethics Committee, Faculty of Medicine, Mansoura University code no: MS.19.12.969. Date: 26/12/2019 and a written informed consent was obtained from all participants before the beginning of the study. Confidentiality was respected and the patient's data were not used for any other aims. The Helsinki Declaration was observed throughout the study's duration.

Statistical analysis

Data were fed to the computer and analysed using IBM SPSS Corp. Released 2013, Version 23. Qualitative data were defined using number and percent. Normally distributed quantitative variables were described in mean ±SD. Abnormally distributed quantitative variables were described in median and range and were compared by Mann Whitney test, which was used to compare quantitative variables in two different groups and by Wilcoxon signed rank test, which was used to compare scores in matched pairs. Spearman's correlation was utilized to assess the validity of association between two variables. Level of significance was considered as 0.05.

RESULTS

The mean age of patients was **31.6 ±10.8 years**. Our study was conducted on **32** female and **8** male patients. There were **14** cases single and **17** married. There were **6** cases with positive family history and **7** females with thyroid disease (**17.5%**). Median duration of disease was **0.2** year. The site of predilection of vitiligo was more (**33 %**) in the upper limb as shown in **table (1)**.

Table (1): Demographic and clinical data of the studied vitiligo patients:

	Studied patients n=40
Age	
Mean ±SD	31.6 ±10.8
Min- max	18-57
Gender	n (%)
Female	32(80)
Male	8(20)
Marital status	n (%)
Single	14(35)
Married	17(42.5)
Divorced	6(15)
Widow	3(7.5)
Family history	6(15)
History of thyroid disease	7(17.5)
Duration of disease in years	
Median	2.0
Min-max	0.2-20.0
Site of lesions	n (%)
Face/neck	10%
Upper limb	33%
Lower limb	20%
Trunk	15%

There was statistically significant difference in comparing VETI scoring before and after treatment with each of Cal/BD and tacrolimus. There was statistically insignificant difference of VETI scoring after treatment when comparing both groups as shown in **table 2 and photos (1-2)**.

Table (2): VETI scoring before versus after treatment in each treatment group:

	Calcipotriol (0.05 mg/g) plus betamethasone Dipropionate (0.5 mg)		Tacrolimus 0.03%		P ^B value	P ^A value
	Before	After	Before	After		
Median	2.5	1.25	3.0	1.5	0.5	0.9
Min-max	0.36-24.0	0.06-24.0	0.36-36.0	0.04-18.0		
P	P<0.001		P<0.001			

P^B: Comparison of score before treatment in 2 groups; P^A: Comparison of score after treatment in 2 groups.



(A)



(B)

Photo (1): A- Bilateral vitiligo lesions before treatment. B- Bilateral vitiligo lesions after treatment with microneedling combined with calcipotriol plus betamethasone dipropionate on right side and microneedling combined with tacrolimus on left side.



Photo (2): A- Bilateral vitiligo lesions in lower limbs before treatment. B- Bilateral vitiligo lesions in lower limbs after treatment with microneedling combined with Cal/BD on side B1 and microneedling combined with tacrolimus on side B2.

There was statistically significant difference in comparing dermoscopic scoring before treatment and after treatment with each of Cal/BD and tacrolimus. There was statistically significant difference in comparing dermoscopic scoring after treatment with Cal/BD versus treatment with tacrolimus as shown in **table 3 and photos (3-4)**.

Table (3): Dermoscopic scoring before versus after treatment in each treatment group:

	Calcipotriol plus betamethasone dipropionate		Tacrolimus		p ^B value	p ^A value
	Before	After	Before	After		
Median	2.0	4.0	2.0	3	1.0	0.04*
Min-max	1-3	2-5	1-3	2-5		
P	<0.001*		0.03*			

P^B: Comparison of score before treatment in 2 groups; P^A: comparison of score after treatment in 2 groups.



Photo (3): A- Dermoscopy of left side of face before treatment. B- Dermoscopy of left side of face after treatment with microneedling combined with tacrolimus.



Photo (4): A-Dermoscopy of right side of face before treatment. B- Dermoscopy of right side of face after treatment with microneedling combined with Cal/BD.

There was statistically positive significant correlation between dermoscopic scoring after treatment with Cal/BD and each of number of sessions and session response. There was statistically positive significant correlation between dermoscopic scoring after treatment with tacrolimus and each of age, number of sessions, session response, and VETI scoring (Table 4).

Table (4): Correlation between dermoscopic scoring after treatment and different factors:

	Calcipotriol (0.05 mg/g) plus betamethasone Dipropionate (0.5 mg) n=40		Tacrolimus (0.03%) n=40	
	R	P	R	P
Age	0.09	0.5	0.3	0.04*
Duration of disease	-0.03	0.8	0.04	0.7
No of sessions	0.3	0.03*	0.4	0.01*
Session response	0.5	0.002*	0.4	0.003*
VETI scoring	0.2	0.2	0.4	0.01*

There was statistically insignificant association between dermoscopy scoring and gender, family history, site of lesion, and thyroid disorders in Cal/BD treated group as shown in table 5.

Table (5): Association between dermoscopy scoring and medical factors in calcipotriol plus betamethasone dipropionate treated group:

	Dermoscopy scoring Median (min-max)	P
Gender		
Male	4 (2-5)	0.5
Female	4 (3-4)	
Family history of vitiligo		
Yes	4(2-4)	0.7
No	4 (2-5)	
Site		
Face/neck	4.0(2-4)	0.3
Upper limb	3.0 (2-5)	
Lower limb	4.0 (3-5)	
Trunk	4.0 (3-5)	
Thyroid disorders		
Yes	3 (3-5)	0.9
No	4 (2-5)	

There was statistically insignificant association between dermoscopy scoring and gender, family history, site of lesion, and thyroid disorders in tacrolimus treated group as shown in table 6.

Table (6): Association between dermoscopy scoring when compared to medical factors in tacrolimus 0.03% treated group:

	Dermoscopy scoring Median (min-max)	P
Gender		
Male	3.0 (2-5)	0.4
Female	3.0 (3-4)	
Family history of vitiligo		
Yes	3.0 (3-4)	0.3
No	3.0 (2-5)	
Site of lesion		
Face/neck	4.0 (3-5)	0.05
Upper limb	3.0 (2-5)	
Trunk	3.0 (3-4)	
Lower limb	3.0 (2-4)	
Thyroid disorders		
Yes	3(2-5)	0.1
No	3(2-5)	

There was statistically positive significant correlation between number of sessions and session response as shown in table 7.

Table (7): Partial correlation between number of sessions and session response:

Control Variables (Type of treatment)	Session response
No sessions	Correlation 0.488
	P <0.001

In our study, minimal side effects were found through sessions as pain in 22.5%, hyperpigmentation 5%, purpura 2.5% and Koebner phenomenon 5%; and 65% of treated patients had no recorded side effects.

DISCUSSION

This study was conducted to compare between the efficiency of microneedling with topical Cal/BD on the right side of any part of the body versus tacrolimus on the left side and the efficacy has documented also by dermoscopy. In present study the mean age of patients was 31.6 ±10.8 years. In agreement with our results, Xing and Xu (12) stated that the mean age was 32.6 years. Female patients were more predominant than male patients where they were 32 female (80%) and 8 male (20%) patients, In agreement with these results Xing and Xu (12) stated that as regards the age of 25 to 45 years, females were more likely to report for vitiligo compared to males. This may be attributed to that females seek early advice because they are more self-conscious about how they look.

In our study, 42.5% of patients were married. In agreement with our results an Indian study conducted

by **Aradhya et al.** ⁽¹³⁾ to assess the impacts of vitiligo on life quality of cases. They demonstrated that 65% of the assessed cases were married which is hand in hand with our study.

Six of our cases had positive family history (15%) of vitiligo. In the same line, **Dave et al.** ⁽¹⁴⁾ reported that 15% of their studied cases were associated with a positive family history, and those patients tended to have a more progressive course compared to the remaining cases. There were seven females with thyroid disease (17.5%) of all vitiligo patients. Comparable findings were recorded by **Vrijman et al.** ⁽¹⁵⁾ who revealed that a thyroid disease was obtained in 15.1% in all patients.

In the present study, good response was identified with right side (A) and left side (B) as regarding the VETI scoring before versus after treatment in each treatment group. On side A there was statistically highly significant decrease in VETI scoring after treatment with microneedling combined with Cal/BD ($p < 0.001$). In accordance with these results **Ibrahim et al.** ⁽¹⁶⁾ stated that the association of microneedling and Cal/BD was demonstrated to be successful.

On side B, there was statistically highly significant decrease in VETI scoring after treatment with microneedling combined with tacrolimus ($p < 0.001$). Similarly, **Ibrahim et al.** ⁽¹⁶⁾ have demonstrated that thirty two percent of patients revealed excellent improvement when using topical tacrolimus in association with microneedling. Combined use of tacrolimus and microneedling has comparable outcomes to its combination with NB-UVB therapy by **Bilal et al.** ⁽¹⁷⁾ without the critical adverse events determined in the later combination. Also, **Mina et al.** ⁽¹⁸⁾ stated that the combined therapy of microneedling with tacrolimus appears to be of great advantage compared to NB-UVB and excimer laser with regard to efficiency, charges, duration of sessions, response time, and safety.

In our study there was statistically insignificant difference of VETI scoring after treatment when comparing both groups. To our knowledge no previous researches studied this before. In the present study our results showed statistically nonsignificant correlation between VETI scoring and age, number of sessions, session response, after treatment with Cal/BD and also after treatment with tacrolimus.

In the current study our results showed statistically nonsignificant correlation between VETI scoring and dermoscopic scoring following treatment with Cal/BD. On the other hand, positively significant correlation was found between VETI scoring and dermoscopic scoring after treatment with tacrolimus. In the present study our results showed statistically nonsignificant association between VETI scoring and the gender, the family history, the site of lesion, the thyroid disease in group treated with Cal/BD and also in the other group treated with tacrolimus.

In our study the combination of microneedling plus both treated groups were found to be effective from clinical view but from dermoscopic view the combined use of microneedling and calcipotriol plus betamethasone was demonstrated to be very efficient than the other treated group ($p 0.001$), ($p=0.03$) respectively and this is the first study demonstrated this efficacy by dermoscopy between microneedling and these two drugs. This may be elucidated by the fact that calcipotriol could stimulate melanin formation via the stimulation of both melanocytes and keratinocytes. Calcipotriene has been demonstrated to have immunosuppressive and immunomodulating characteristics. Receptors for the active form of vitamin D have been demonstrated on keratinocytes, melanocytes, and fibroblasts and on active immunological cells. Melanocytes and keratinocytes within vitiliginous areas displayed defective Ca^{++} uptake. According to this observation, the authors displayed that calcipotriene could be efficient for vitiligo, either alone or in combination and potent immunosuppressive actions of topical corticosteroids could participate in rapid repigmentation inhibiting autoreactive lymphocytes towards melanocytes.

It works in a synergistic manner to restore pigmentation and inhibit the immune system. Vitamin D influences the intracellular pathways that corticosteroids share, improving the clinical outcome for vitiligo cases ⁽⁸⁾. It also has a main role in the context of Ca^{++} homeostasis by decreasing abnormal Ca^{++} influx into the melanocytes and thus induces melanogenesis ⁽¹⁹⁾. Betamethasone dipropionate improves the protective and anti-destructive mechanism of melanocytes, suppresses the localized immunological changes as a result permitting the melanocytes to reactivate and repigment ⁽²⁰⁾. This was in accordance with similar results by **Ibrahim et al.** ⁽¹⁶⁾ who stated that he combined use of microneedling and Cal/BD was shown to be very efficient compared to the combined use of microneedling with tacrolimus. This study supported dermoscopic view in our present study.

In the present study the predominant finding by dermoscopy in recovery of pigmentation was perifollicular pigmentation in group treated with microneedling with tacrolimus and also in group treated with microneedling with Cal/BD but with telangiectasia. In agreement with our study, **Wang et al.** ⁽²¹⁾ dermoscopy was applied to detect the recovery of pigmentation after combination therapy of tacrolimus ointment plus 308 nm excimer laser in vitiligo cases and the most important result was residual perifollicular pigmentation, whereas a limited number of cases had perilesional hyperpigmentation. In agreement with previous studies, **Abd Elazim et al.** ⁽²²⁾ stated that dermoscopy assessment of the ninety responsive lesions also demonstrated perifollicular diffuse pigmentation and was the most frequent pattern of repigmentation. Perifollicular pigment spread was

the highest in group treated with follicular unit extraction grafting technique (FUE) combined with topical calcipotriol betamethasone dipropionate followed by group treated with (FUE) combined with NB-UVB and lastly group treated with (FUE) alone.

All adverse events noticed in the current study were minor and involved pain, erythema which diminishes following 24 hours, and exfoliations disappeared within few days, only one patient had purpura subside after one week. Such adverse events effects didn't vary from those formerly recorded in studies for side A (8, 23) or side B (17, 24). In contrast, a study by **Sethi et al.** (25) revealed extensive repigmentation in 56.7% of cases at 4 months following combination of 5-FU with dermabrasion.

To properly evaluate the strength of the current procedure; we extended the follow up period for another three months after the end of the treatment protocol. We demonstrated that the repigmentation acquired in both lesions A and B was stable in all cases except two cases recurrence occurred with the two drugs on both sides. In other previous study reported by **Abd Elazim et al.** (22) they stated that the follicular unit extraction grafting technique (FUE) combined with topical calcipotriol plus betamethasone dipropionate (CBD) had the upper hand of over the other two groups (FUE grafting alone or combined with NB-UVB) with regard to the production of better grades of repigmentation, better colour match, and significant reduction in the size. Additionally, topical CBD combination targets local immune response, serves on specific T-cell stimulation, and affects melanocyte maturation and differentiation. In previous study reported by **Bakr et al.** (26) they stated that in a group of patients managed by topical calcipotriol together with the fractional CO₂ laser, ten percent of cases revealed excellent improvement and sixty percent revealed good and very good outcomes. In another previous study used combined excimer laser and topical calcipotriol in the context of vitiligo management, a repigmentation rate of 65% was recorded (27). Additionally, **Ibrahim et al.** (16) demonstrated that sixty percent of the cases demonstrated excellent improvement following the use of microneedling together with topical calcipotriol (0.05 mg/g) plus betamethasone (0.5 mg) ointment. This could elucidate the fact that adding topical steroids in their study is still the best standard and efficient management for vitiligo cases (26).

In previous study reported by **Mina et al.** (18) they stated that forty percent of the patches managed with tacrolimus accomplished marked response (repigmentation>50%), whereas fifty percent of patches managed with tacrolimus combined with the 308 nm excimer laser demonstrated response (>75% repigmentation) as revealed by **Kawalek et al.** (28) who demonstrated that the combined therapy was faster and more efficient compared to excimer laser only. Also, **Park et al.** (29) displayed that the combined use of

tacrolimus and excimer laser was associated with a significant improvement compared to tacrolimus or excimer laser individually. In previous study reported by **Bakr et al.** (26) they stated that after using the combined therapy of topical tacrolimus and fractional CO₂ laser, thirty percent of cases demonstrated excellent improvement (>75% repigmentation).

CONCLUSIONS

Microneedling combined with Cal/BD ointment or combined with tacrolimus ointment seems to be safe and effective therapeutic options in vitiligo treatment. Dermoscopically the combination with Cal/BD has a statistically higher outcome compared with the combination with tacrolimus ointment.

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